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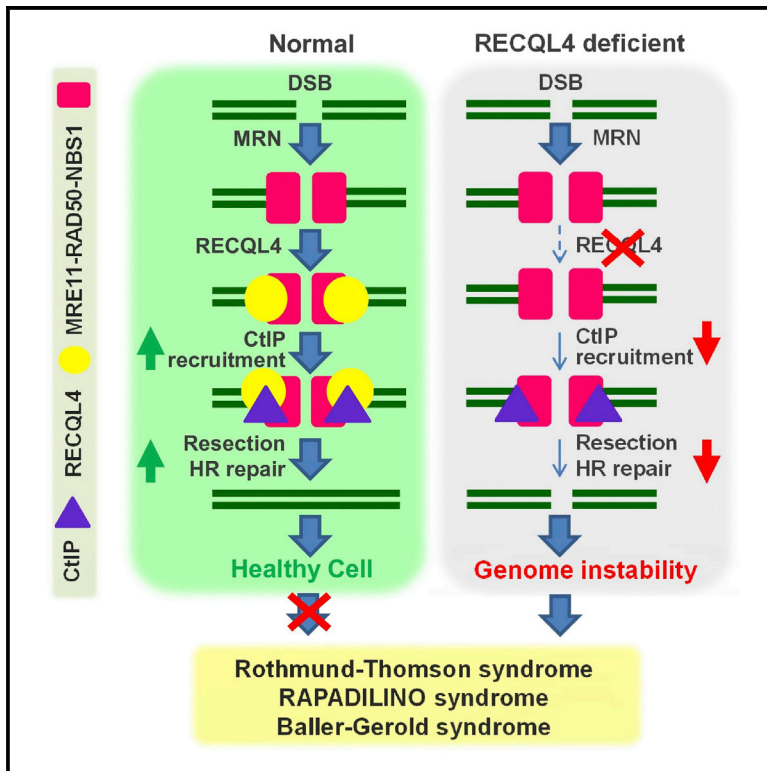
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Graphical Abstract



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In Brief

RECQL4, a RecQ helicase mutated in Rothmund-Thomson syndrome, is a guardian of genome stability and repairs DNA, but the underlying mechanisms remain unclear. Lu et al. show that RECQL4 plays a role in homologous recombination repair of DNA double-strand breaks (DSBs). RECQL4 promotes 5' DNA end resection through the MRE11-RAD50-NBS1 and CtIP complexes.

Highlights

- RECQL4 promotes 5' end resection at DSBs
- RECQL4 recruitment to DSBs depends on MRE11
- RECQL4 promotes recruitment of CtIP to DSBs
- RECQL4 helicase activity is required for 5' DNA end resection



RECQL4 Promotes DNA End Resection in Repair of DNA Double-Strand Breaks

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SUMMARY

The RecQ helicase RECQL4, mutated in Rothmund-Thomson syndrome, regulates genome stability, aging, and cancer. Here, we identify a crucial role for RECQL4 in DNA end resection, which is the initial and an essential step of homologous recombination (HR)-dependent DNA double-strand break repair (DSBR). Depletion of RECQL4 severely reduces HR-mediated repair and 5' end resection in vivo. RECQL4 physically interacts with MRE11-RAD50-NBS1 (MRN), which senses DSBs and initiates DNA end resection with CtIP. The MRE11 exonuclease regulates the retention of RECQL4 at laser-induced DSBs. RECQL4 also directly interacts with CtIP via its N-terminal domain and promotes CtIP recruitment to the MRN complex at DSBs. Moreover, inactivation of RECQL4's helicase activity impairs DNA end processing and HR-dependent DSBR without affecting its interaction with MRE11 and CtIP, suggesting an important role for RECQL4's unwinding activity in the process. Thus, we report that RECQL4 is an important participant in HR-dependent DSBR.

INTRODUCTION

DNA double-strand breaks (DSBs) are generated by exogenous stress, endogenous replication, and programmed recombination events. Improperly repaired DSBs can lead to genome instability, chromosomal rearrangements, and/or cell death (Symington, 2014). DSBs are usually repaired by one of two major pathways: homologous recombination (HR) and non-homologous end joining (NHEJ) (Aparicio et al., 2014). HR-dependent DSBR is mostly error free, but it requires a sister or non-sister chromatid as template and is only active during the S and G2 phases of the cell cycle. In contrast, NHEJ-dependent DSBR is error prone, DNA template-independent, and active during all phases of the cell cycle.

HR-dependent DSBR is initiated by 5' end resection of the DSBs, which generates 3' protruding single-strand DNA (ssDNA) tails (Chen et al., 2013; Zhu et al., 2008). RPA coats the ssDNA, and then RAD51 replaces RPA to promote strand invasion. This is followed by repair synthesis, dissolution, and resolution of Holliday junctions and ligation of the ends (Prakash et al., 2015). It is generally considered that DNA end resection occurs in two steps (Cejka et al., 2010; Gravel et al., 2008; Mimitou and Symington, 2008; Nimmonkar et al., 2011; Niu et al., 2010; Zhu et al., 2008). The first step is the initial resection by Mre11-Rad50-Xrs2 (MRX) and Sae2 at the DSB in yeast (Cannavo and Cejka, 2014; Mimitou and Symington, 2008) or by MRE11-RAD50-NBS1 (MRN) and CtIP (CtBP-interacting protein) in human cells (Sartori et al., 2007; You et al., 2009). This is followed by extensive resection by either exonuclease1 (EXO1) or DNA2/BLM/TOP3/RMI1/2 (Dna2/Sgs1/Top3/Rmi1 in yeast) (Cejka et al., 2010; Gravel et al., 2008; Mimitou and Symington, 2008; Nimmonkar et al., 2008, 2011; Niu et al., 2010; Zhu et al., 2008).

RECQL4 is one of five RecQ helicase proteins in mammalian cells. Defects in human RECQL4 are associated with three genetic diseases: Rothmund-Thomson syndrome (RTS), RAPADILINO, and Baller-Gerold syndrome (Siitonen et al., 2009) as well as several cancers (Fang et al., 2013; Lu et al., 2014b; Su et al., 2010). It is well established that RECQL4 is required for the assembly of the DNA replication initiation machinery (Im et al., 2009; Sangrithi et al., 2005; Xu et al., 2009). However, the role of RECQL4 in DNA repair is less clear (Croteau et al., 2014). Lack of RECQL4 increases persistent DNA damage and triggers cellular senescence in human and mouse primary fibroblasts (Lu et al., 2014a). RECQL4 is recruited to laser-induced DSBs and RTS fibroblasts are sensitive to ionizing radiation (IR), suggesting that RECQL4 plays a role in DSBR (Singh et al., 2010). Recently, we showed that depletion of RECQL4 inhibits NHEJ in U2OS cells (Shamanna et al., 2014). Nevertheless, RECQL4 is highly expressed during S phase (Singh et al., 2012; Xu et al., 2009), when HR-dependent DSBR dominates. Thus, we explore the possibility that RECQL4 also plays a role in HR-dependent DSBR. We find that RECQL4 promotes DNA end resection and HR-dependent DSBR by stimulating the association of CtIP with MRN at DSBs and that the helicase activity of RECQL4 is necessary for DNA end resection. Together, these

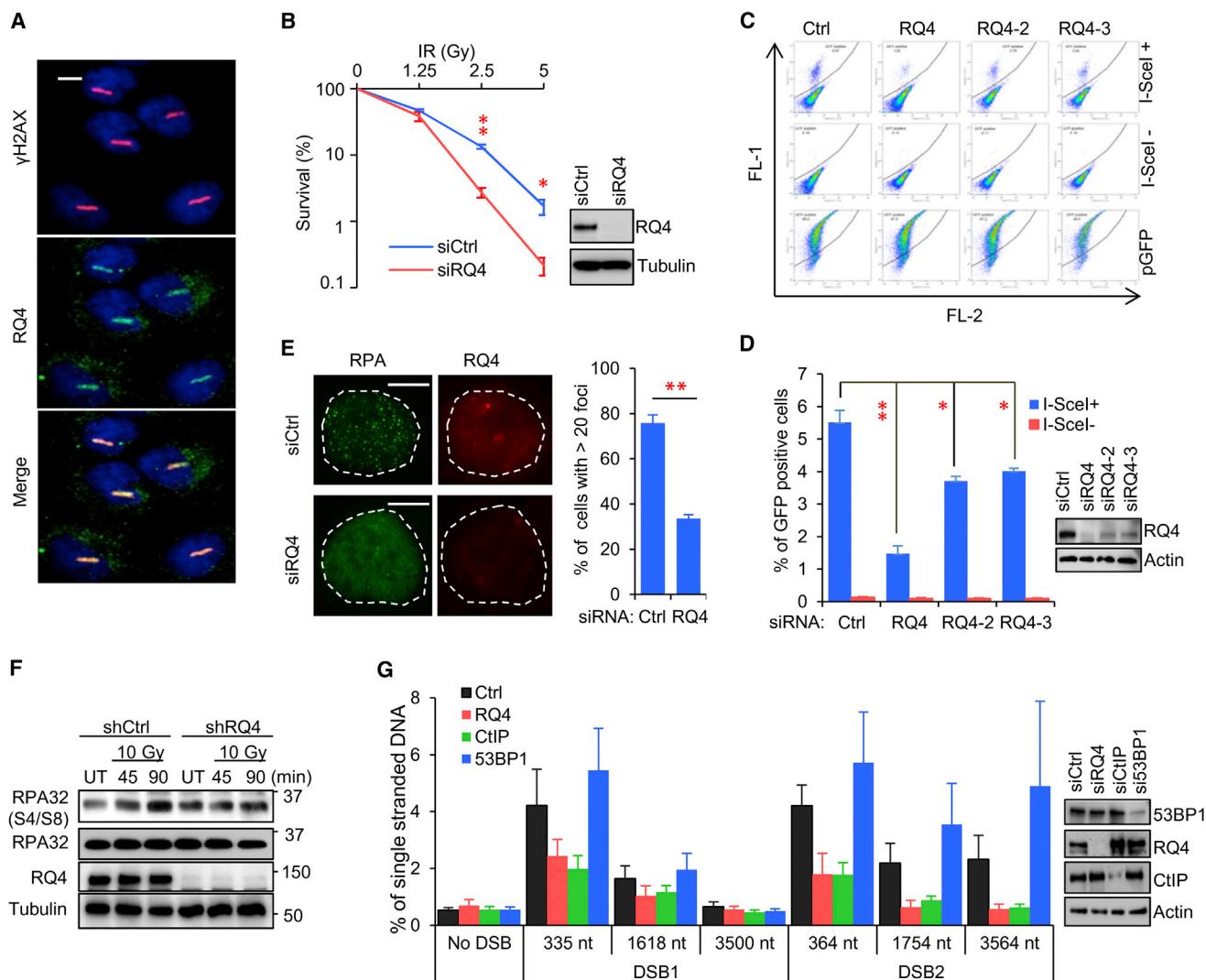


Figure 1. RECQL4 Is Required for HR-Mediated Repair and DNA End Resection

(A) Co-localization of endogenous RECQL4 and γ H2AX at laser-induced DSB tracks. Scale bar represents 10 μ m.

(B) Clonogenic survival of siRQ4-transfected U2OS cells treated with γ radiation.

(C) Representative dot-plot images of DR-GFP U2OS cells showing *in vivo* HR.

(D) Quantification of HR repair.

(E) RPA foci in U2OS cells treated with control or RECQL4 siRNA 1 hr after 10 Gy IR. Scale bar represents 10 μ m.

(F) IR-induced RPA32 phosphorylation on serine 4 and 8. U2OS cells expressing control or RECQL4 shRNA were exposed to 10 Gy of IR then allowed to recover for the indicated time. UT, untreated.

(G) Quantification of ssDNA generated by 5' end resection at two AsiSI-induced DSBs in AID-DlvA U2OS cells.

All graphs show mean \pm SEM from at least three biological repeats. p values (*p < 0.05; **p < 0.01) was determined by Student's t test. See also Figures S1 and S2.

findings suggest that RECQL4 plays an important role in the DNA end resection step of HR-mediated DSB repair in human cells.

RESULTS

RECQL4 Promotes DNA End Resection during HR-Dependent DSB Repair

Endogenous RECQL4 co-localized with γ H2AX at laser-induced DSBs in U2OS cells (Figure 1A) and depletion of RECQL4 caused

U2OS and HeLa cells to be significantly more sensitive to IR (Figures 1B and S1A). Since DSB repair pathway choice is cell-cycle regulated (Aparicio et al., 2014), we first examined the effect of RECQL4 depletion on cell-cycle progression. Knockdown of RECQL4 did not perturb cell-cycle progression significantly in U2OS or HEK293T cells and did not alter expression of cell-cycle marker proteins Cyclin A and Cyclin D1 (Figure S2), which is consistent with a previous finding in HEK293 cells (Park et al., 2006).

The role of RECQL4 in HR-dependent DSB repair was then investigated in DR-GFP U2OS cells, which can be scored for efficiency of HR-mediated repair of an I-SceI endonuclease-induced DSB by measuring the fraction of GFP-positive cells (Pierce et al., 1999; Wang et al., 2014). DR-GFP U2OS cells were transfected with one of three RECQL4-targeted small interfering RNAs (siRNAs). The efficiency of RECQL4 knockdown was about 90%, 60%, and 50% for siRQ4, siRQ4-2, and siRQ4-3, respectively (Figure 1D). Given that siRQ4 produced the greatest knockdown it was used in all subsequent experiments. Depletion of RECQL4 by siRQ4 significantly reduced the proportion of GFP-positive cells by 73%, from 5.5% in control cells to 1.47% in knockdown cells (Figures 1C and 1D), suggesting that RECQL4 plays a crucial role in HR-dependent DSB repair. The other two siRNAs, siRQ4-2 and siRQ4-3, also significantly reduced the proportions of GFP-positive cells to 3.71% and 4.0%, respectively (Figures 1C and 1D). These data show that knockdown efficiency of RECQL4 correlates with a decrease of HR-mediated DSB repair.

RECQL4 is rapidly recruited to laser-induced DSBs where it is retained for a short time (Singh et al., 2010). Thus, we speculated that it plays a role in the early stages of HR repair. As mentioned above, the first step of HR-mediated DSB repair is 5'-3' end resection of the DSBs to generate 3' protruding ssDNA tails, which are rapidly coated by RPA to form a nuclease-resistant protective protein-DNA filament (Chen et al., 2013). We then examined RPA foci formation in RECQL4-depleted cells. After exposure to 10 Gy of IR, the fraction of cells with >20 RPA foci was ~33.6% in the siRQ4-treated U2OS cells, significantly less than 75.8% in the control cells (Figure 1E). Depletion of RECQL4 also repressed RPA foci formation after IR in HeLa cells (Figure S1B). Consistent with these results, the abundance of phosphorylated RPA32 on serine 4 and serine 8, a marker of ssDNA-bound RPA (Shao et al., 1999), was increased in IR-treated control U2OS and HEK293T cells, but not in RECQL4 knockdown cells (Figures 1F and S1C).

AID-DivA U2OS cells have been used to directly quantify ssDNA generated by 5' end resection at two AsiSI-induced DSBs (Aymard et al., 2014). With addition of 4-hydroxytamoxifen (4-OHT), the AsiSI endonuclease fused to an estrogen receptor ligand binding domain translocates from the cytoplasm to the nucleus to induce DSBs (Aymard et al., 2014). Genomic DNA from these cells was prepared and analyzed for ssDNA at two DSBs by TaqMan qPCR, as previously described (Aymard et al., 2014; Zhou et al., 2014). Consistent with a previous report (Zhou et al., 2014), we observed notably lower ssDNA in cells treated with CtIP siRNA because CtIP stimulates DNA end resection (Sartori et al., 2007). On the contrary, depletion of 53BP1 increased the amount of ssDNA (Figure 1G), since 53BP1 inhibits resection (Bunting et al., 2010). To investigate the role of RECQL4 in 5' end resection, ssDNA content was measured in siRQ4-transfected AID-DivA U2OS cells. Interestingly, the amount of ssDNA generated at position 335 nt from DSB1 was 40.2% lower than that in siCtrl-treated cells, and similar to the reduction caused by CtIP depletion. At DSB2, depletion of RECQL4 reduced ssDNA content by 57.2%, 70.1%, and 75.2% at positions 364, 1,754, and 3,574 nt, respectively. Together, these results demonstrate

that RECQL4 is important for HR by promoting 5' end resection of DSBs.

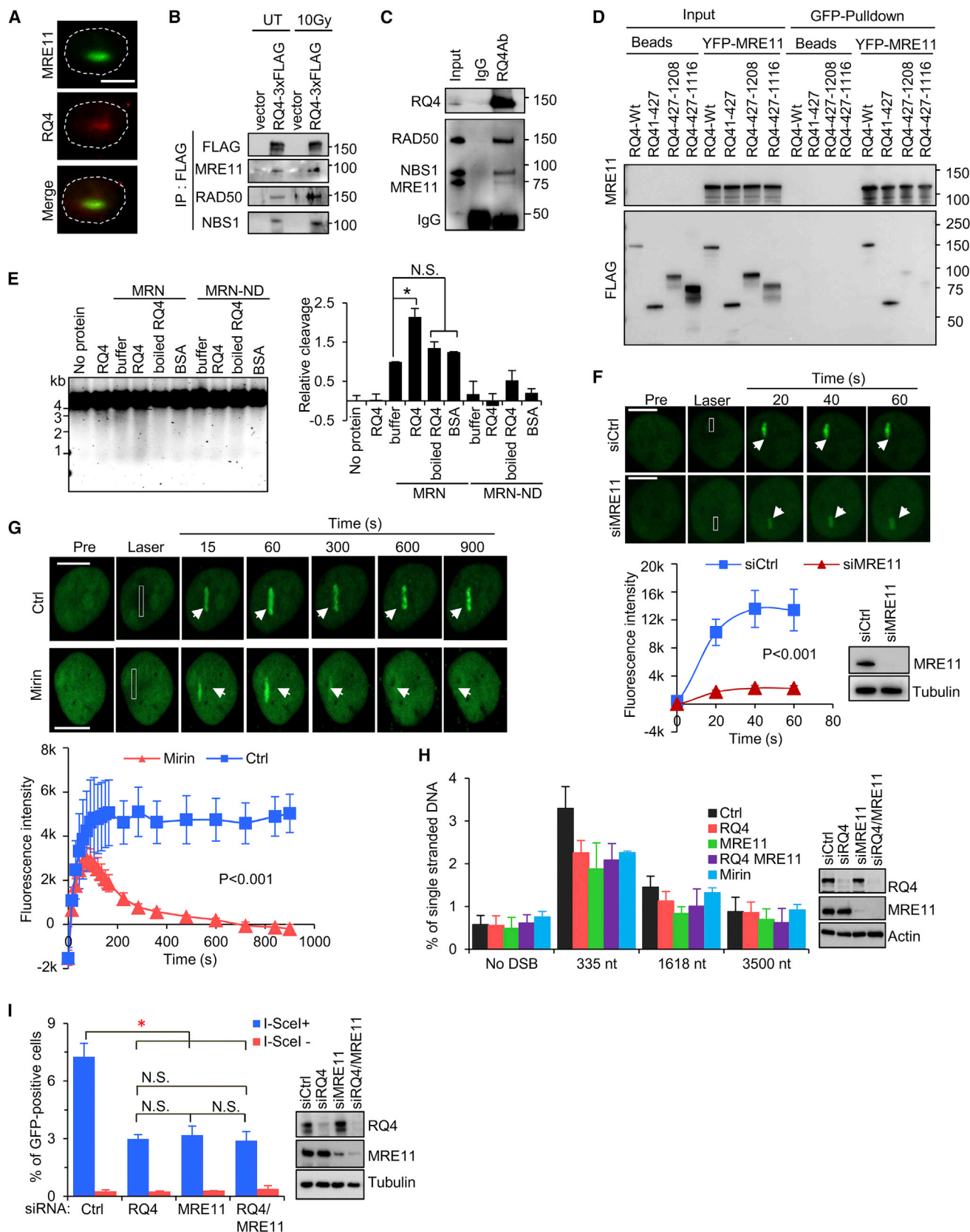
Retention of RECQL4 at DSBs Depends on MRE11

To explore the function of RECQL4 in 5' end resection, we used mass spectrometry to analyze proteins captured by co-immunoprecipitation (IP) with RECQL4-3xFLAG from irradiated HEK293T cells in the presence of benzonase. MRN components MRE11 and RAD50 and other DNA resection proteins BLM, EXO1, and DNA2 were identified (Figure S3A; Table S1), and their interactions with RECQL4 were independently confirmed by IP with GFP-RECQL4 in the presence of benzonase (Figure S3B) or ethidium bromide (Figure S3C). We found that RECQL4 co-localized with MRE11 at DSBs and that the RECQL4-MRN interaction is stimulated by IR (Figures 2A and 2B). Purified recombinant RECQL4 also immunoprecipitated recombinant MRE11, RAD50, and NBS1 (Figure 2C), indicating complex formation between RECQL4 and MRN. To map the interaction region of RECQL4 with MRE11, purified RECQL4-3xFLAG and truncation fragments were incubated with purified YFP-MRE11 bound to GFP agarose beads. YFP-MRE11 pulled down full-length and the N-terminal domain of RECQL4 (Figure 2D), indicating that the N-terminal fragment of RECQL4 is responsible for the interaction with MRE11. To determine whether the interaction between RECQL4 and MRN is functional, we measured the nuclease activity of MRN on closed-circular single-strand PhiX174 DNA in the presence of RECQL4 *in vitro* as previously reported (Sartori et al., 2007). Wild-type MRN and nuclease-dead MRN-ND (H129L/D130V) (Stracker et al., 2002) as well as RECQL4 and its helicase-dead mutant RQ4KM were used (Figure S4A). We found that RECQL4 slightly stimulated the nuclease activity of MRN on closed-circular single-strand PhiX174 DNA (Figure 2E).

Both RECQL4 and MRE11 are rapidly recruited to DSB (Haince et al., 2008; Singh et al., 2010), and thus we evaluated whether RECQL4 and MRE11 affected each other's recruitment to DNA damage. GFP-RECQL4 was recruited significantly less to laser-induced DSBs in siMRE11-treated U2OS cells than in control cells (Figure 2F), and there is less chromatin-bound RECQL4 in siMRE11-treated U2OS cells than in control cells after IR (Figure S4B). However, depletion of RECQL4 did not affect the recruitment of YFP-MRE11 to laser-induced DSB (Figure S4C), suggesting that recruitment of RECQL4 to DSBs requires MRE11, but not vice versa.

Given that MRE11 nuclease regulates the pathway choice between NHEJ and HR (Shibata et al., 2014), the dynamics of GFP-RECQL4 recruitment was also evaluated in cells exposed to mirin, which specifically inhibits the MRE11 exonuclease but does not inhibit MRN complex formation (Dupré et al., 2008). RECQL4 was still rapidly recruited to laser-induced DSBs in mirin-treated cells (Figure 2G), indicating that the recruitment of RECQL4 does not depend on the exonuclease activity of MRE11. However, GFP-RECQL4 was retained at DSBs for a significantly shorter time after mirin treatment (Figure 2G). This suggests that retention of RECQL4 at DSBs is regulated by MRE11 nuclease activity.

When ssDNA was measured at AsiSI-induced DSB in AID-DivA U2OS cells pre-treated with mirin, siRQ4 or siMRE11, a



(legend on next page)

lower amount of ssDNA was detected (Figure 2H). However, the effect was not additive (Figure 2H). Using the DR-GFP reporter system, it was observed that pre-treatment with siRQ4 or siMRE11 significantly reduced HR-mediated DSB, but the effect was also not additive (Figure 2I). These results suggest that RECQL4 functions downstream of MRN to promote DNA 5' end resection and HR-dependent DSB.

RECQL4 Promotes Recruitment of CtIP to DSBs

CtIP is required for initiation of MRN-catalyzed 5' end resection at DSBs (Chen et al., 2008; Sartori et al., 2007; Yuan and Chen, 2009). Here, we found that RECQL4 co-localized with CtIP at laser-induced DSBs (Figure 3A) and interacted with CtIP in irradiated HEK293T cells (Figures 3B, S3B, and S3C). The interaction between CtIP and RECQL4 appeared to be stronger in IR-treated cells (Figure 3B). CoIP of recombinant RECQL4 and CtIP suggests that RECQL4 interacts directly with CtIP (Figure 3C), and the N terminus of RECQL4 was mapped as the interacting region with CtIP (Figure 3D).

Recruitment of RECQL4 reaches its peak about 1 min after laser damage (Figure 2G), while CtIP needs much longer (Wang et al., 2013). Considering the direct interaction between RECQL4 and CtIP, it is possible that RECQL4 promotes CtIP recruitment to DSBs. To test this hypothesis, we first measured the abundance of chromatin-bound CtIP in control and RECQL4 knockdown U2OS cells after IR and found that IR increased chromatin-bound CtIP in the control cells but not in RECQL4-depleted cells (Figure 3E). Interestingly, more mobility shift of chromatin-bound CtIP was detected in control cells than in RECQL4-depleted cells after IR (Figure 3E), indicating that RECQL4 promotes IR-induced posttranslational modification of CtIP. Also, IR-treated control U2OS cells had an average of 22.6 GFP-CtIP foci per cell, significantly higher than that in siRQ4-treated cells (Figure 3F). Furthermore, in the RECQL4 knockdown U2OS cells, recruitment of GFP-CtIP was significantly slower and less efficient than that in control cells (Figure 3G). Together these data suggest that RECQL4 promotes stable CtIP recruitment to DSBs.

Given that RECQL4 promotes recruitment of CtIP to DSBs, we asked whether RECQL4 is required for MRN-CtIP complex formation after IR. Pull-down assays were conducted in control

and RECQL4 knockdown HEK293T cells expressing YFP-MRE11 or GFP-CtIP. Expression levels of MRE11, RAD50, NBS1, and CtIP proteins were similar in control and RECQL4 knockdown cells (input of Figure 3H). Cell-cycle status was not significantly different between RECQL4-depleted and control HEK293T cells (Figures S2D and S2E). IP of YFP-MRE11 efficiently pulled down similar amounts of RAD50 and NBS1 from control and RECQL4 knockdown cells. In contrast, the interaction between MRE11 and CtIP was inhibited by knockdown of RECQL4 (Figure 3H). In the reverse experiments with GFP-CtIP-expressing cells, GFP-CtIP efficiently co-immunoprecipitated MRE11, RAD50, and NBS1 from control cells but much less from RECQL4 knockdown cells (Figure 3H). These data are consistent with the idea that RECQL4 promotes the interaction between MRN and CtIP in human cells.

Depletion of RECQL4 or CtIP significantly reduced ssDNA generation at DSB1 in AID-DivA cells (Figure 3I). However, there were no differences among RECQL4 or CtIP-depleted cells and RECQL4/CtIP double-knockdown cells. A similar result was obtained from the experiments measuring the HR efficiency (Figure 3J). These results imply that RECQL4 and CtIP both play a role in HR-dependent DSB and that RECQL4 promotes recruitment of CtIP to DSBs.

BLM and EXO1 Act Downstream of RECQL4 during HR-Mediated DSB

5' resection, initiated by the MRN-CtIP complex, is extended by BLM/DNA2 and EXO1 via two alternative pathways (Cejka, 2015; Symington, 2014). Recruitment of both BLM and EXO1 to DSBs requires CtIP (Wang et al., 2013). Since RECQL4 promotes CtIP recruitment to DSBs, we tested whether removal of RECQL4 could result in failure of the two extensive resection pathways. We found that BLM, DNA2, and EXO1 interact with RECQL4 in irradiated HEK293T cells (Figure S3). In addition, the retention of GFP-BLM at DSBs was reduced in U2OS cells after RECQL4 knockdown (Figure 4A), suggesting that RECQL4 stimulates retention of BLM at IR-induced DSBs. In addition, knockdown of BLM, RECQL4, or both inhibited 5' resection to a similar extent at DSB1 in AID-DivA U2OS cells (Figure 4B). In the HR assay, knockdown of BLM, RECQL4, or both significantly reduced HR by 40%, 60%, or 60%, respectively (Figure 4C). These findings

Figure 2. MRE11 Mediates Recruitment of RECQL4 to DSBs to Promote HR Repair

- (A) Co-localization of endogenous RECQL4 and MRE11 at laser-induced DSB tracks in U2OS cells. Scale bar represents 10 μ m.
- (B) RECQL4 interacts with MRN complex in vivo. FLAG-IP was carried out using extracts prepared from vector and RQ4-3xFLAG expressing HEK293T cells treated with a 10-Gy IR and recovered for 10 min.
- (C) CoIP of recombinant MRE11, RAD50, and NBS1 with RECQL4.
- (D) N-terminal domain of RECQL4 interacts with MRE11.
- (E) Nuclease of MRN is stimulated by RECQL4 on closed circular single-strand PhiX174 DNA. MRN (20 nM) or the nuclease-dead mutant, MRN-ND, was incubated with 20 nM RECQL4, boiled RECQL4, or BSA. Buffer, nuclease reaction buffer.
- (F) MRE11 promotes RECQL4 recruitment to DSBs in U2OS cells. The recruitment of GFP-RECQL4 to DSB tracks, generated with a 435-nm laser, was monitored in the control and MRE11-depleted U2OS cells, and the fluorescence intensity was quantified. $n = 27$. Scale bar represents 10 μ m.
- (G) Retention of RECQL4 at DSBs depends on the exonuclease activity of MRE11. U2OS cells were treated with 100 μ M mirin. Graphic quantification below images, $n = 21$. Scale bar represents 10 μ m.
- (H) RECQL4 and MRE11 regulate resection at DSBs. Bar graph showing percent of ssDNA content generated at DSB1 in siRNA- or mirin-treated cells. Error bars represent SEM from four biological repeats.
- (I) HR repair assay and western blots from RECQL4 and MRE11 knockdown DR-GFP cells.
- All data are presented as mean \pm SEM from at least three independent experiments with p value calculated with Student's t test. * $p < 0.05$. See also Figures S3 and S4 and Table S1.

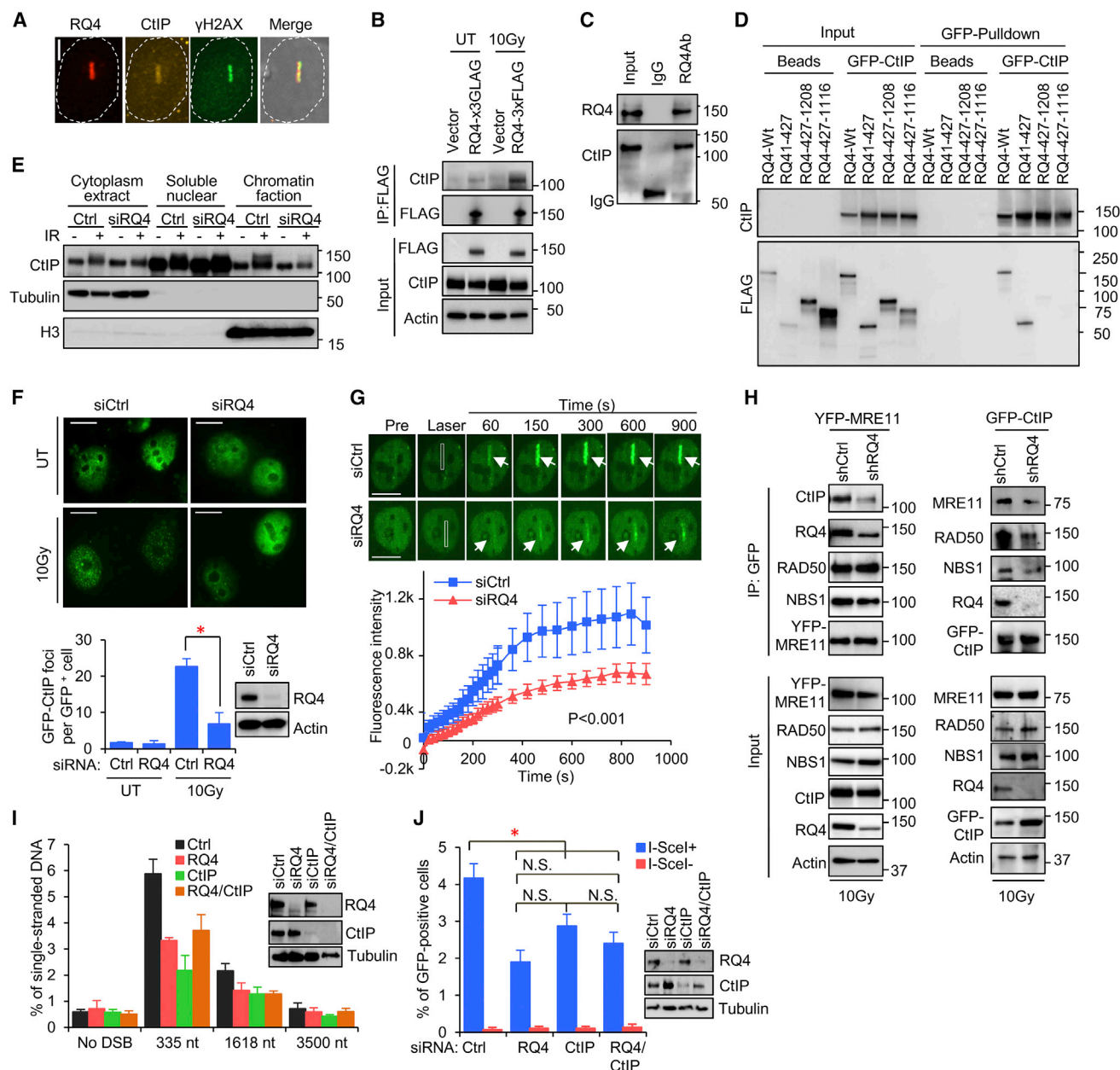


Figure 3. RECQL4 Promotes CtIP Recruitment to DSBs for DNA End Resection and HR Repair

(A) Co-localization of endogenous RECQL4 and CtIP at laser-induced DSB tracks. Scale bar represents 5 μ m.

(B) CoIP of CtIP with RECQL4 in response to IR.

(C) In vitro colP analysis of recombinant RECQL4 and CtIP.

(D) The N terminus of RECQL4 interacts with CtIP.

(E) Subcellular distribution of CtIP in control and RECQL4-depleted U2OS cells 10 min after IR.

(F) GFP-CtIP foci in the control and RECQL4-depleted U2OS cells 30 min after IR. Scale bar represents 10 μ m.

(G) Recruitment of GFP-CtIP to DSB tracks in control and RECQL4-depleted U2OS cells. n = 29. Scale bar represents 10 μ m.

(H) RECQL4 supports the interaction between MRN and CtIP. Western analysis of indicated proteins pulled down with YFP-MRE11 or GFP-CtIP from control and RECQL4 knockdown HEK293T cells 10 min after IR.

(I) Quantification of ssDNA generated at DSB1 in AID-DiVa U2OS cells after knockdown of RECQL4 and CtIP.

(J) HR repair assay after knockdown of RECQL4 and CtIP in DR-GFP U2OS cells.

Error bars for (I) and (J) represent SEM from three independent experiments. The IR dose is 10 Gy. See also Figures S2 and S3 and Table S1.

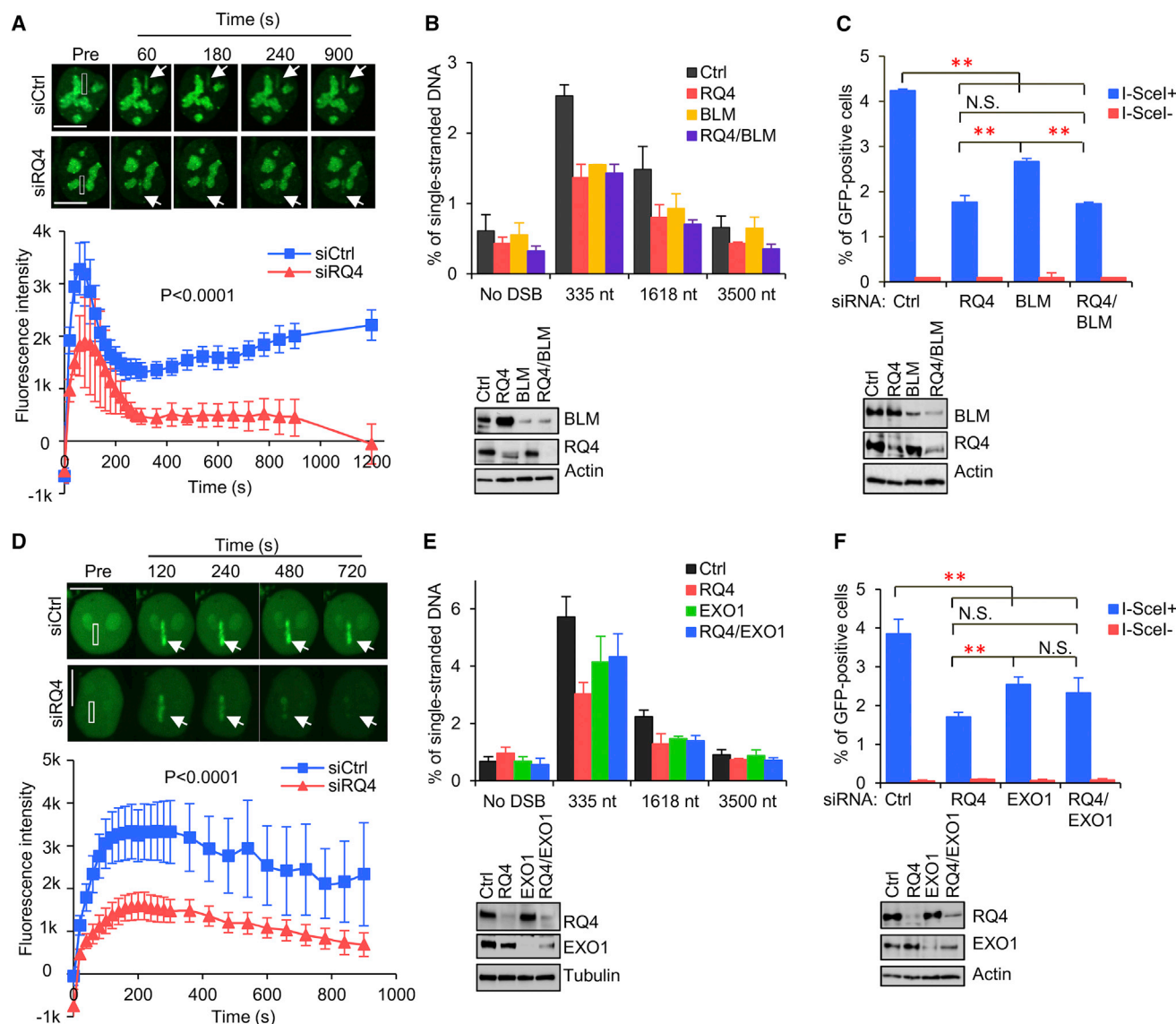


Figure 4. RECQL4 Promotes Recruitment of BLM and EXO1 to Laser-Induced DSBs

(A) Recruitment of GFP-BLM in control and RECQL4-depleted U2OS cells. $n = 22$. Scale bar represents 10 μ m. (B) Quantification of ssDNA content generated at DSB1 in AID-DivA U2OS cells depleted for RECQL4 and BLM. (C) Quantification of GFP-positive cells from the HR repair in control, RECQL4, and BLM knockdown cells. (D) Recruitment of GFP-EXO1 in control and RECQL4 knockdown U2OS cells. $n = 27$. Scale bar represents 10 μ m. (E) Quantification of the ssDNA generated from resection at DSB1 in cells with knockdown for RECQL4 and EXO1. (F) HR assay from EXO1 and RECQL4 knockdown DR-GFP cells. Data are presented as mean \pm SEM from three biological repeats. See also Figure S3 and Table S1.

suggest that RECQL4 promotes retention of BLM at DSBs to stimulate HR.

We also evaluated the impact of RECQL4 loss on EXO1-mediated resection. RECQL4 co-localized with EXO1 at laser-induced DSBs in U2OS cells (Figure S3E) and co-immunoprecipitated endogenous EXO1 in irradiated U2OS cells but not in untreated cells (Figure S3F). GFP-EXO1 was rapidly recruited to DSBs in U2OS cells, but significantly more slowly in siRQ4-treated U2OS cells (Figure 4D), indicating that RECQL4 also pro-

motes EXO1 function at DSBs. Knockdown of EXO1 reduced the amount of 5'-end-resected DSBs in AID-DivA U2OS cells (Figure 4E), which is consistent with previous findings (Zhou et al., 2014). However, EXO1 and RECQL4 double knockdown was not additive in resection of DSBs (Figure 4E). EXO1 knockdown reduced HR by about 31.4% but did not significantly exacerbate the reduction of HR in combination with RECQL4 depletion (Figure 4F). These data support a model in which RECQL4 acts upstream of DNA2/BLM and EXO1 in HR-dependent DSB repair.

RECQL4 Helicase Activity Is Required for DNA End Resection during HR-Mediated Repair

The RecQ proteins share a conserved RecQ helicase domain and possess 3'-5' DNA unwinding activity (Croteau et al., 2014). The helicase activity of human RECQL4 is weak compared to the others in vitro (Rossi et al., 2010; Xu and Liu, 2009). However, mutations in the helicase domain have been identified in many reported RECQL4-associated syndrome patients (Siitonen et al., 2009), indicating the importance of the helicase domain in vivo. To explore whether RECQL4 helicase activity is involved in DNA end resection and HR repair, we transfected siRQ4-resistant plasmids to ectopically express 3xFLAG-tagged wild-type (WT) RECQL4 or the helicase-dead mutant RECQL4-KM in siRQ4-treated AID-DivA U2OS or DR-GFP U2OS cells. Western blots showed that endogenous RECQL4 was depleted by siRQ4 and that 3xFLAG-tagged RECQL4 and RECQL4-KM were expressed in AID-DivA U2OS cells (Figure 5A). Depletion of RECQL4 resulted in a reduction of ssDNA generated by DNA resection at the DSB1 site, and overexpression of RECQL4-3xFLAG completely restored the loss of 5' end resection in siRQ4-transfected cells, whereas overexpression of RECQL4-KM-3xFLAG did not (Figure 5A). These results indicate that the RECQL4 helicase activity is required for DNA end resection.

In the HR repair assay, DSBs were generated by transfection of I-SceI-expressing plasmid into DR-GFP U2OS cells (Pierce et al., 1999). To reduce competition with I-SceI-expressing plasmid, the amount of pCMVtag4A-RQ4-siR, pCMVtag4A-RQ4KM-siR or vector was reduced to 0.5 μ g for 2×10^6 cells, which resulted in a low expression level of 3xFLAG-tagged RECQL4 and the mutant. However, this level of RECQL4-3xFLAG still significantly increased the percentage of GFP-positive cells, depleted for endogenous RECQL4 (Figure 5B). This is consistent with our observation that RECQL4 levels correlate with HR repair (Figures 1C and 1D). The RECQL4-KM-3xFLAG expression was higher than that of WT RECQL4 but did not significantly rescue the loss of HR repair after RECQL4 depletion. These data suggest that the RECQL4's helicase activity is important for DNA end resection and HR-dependent DSBR.

Given that RECQL4 promotes complex formation between CtIP and MRN by interacting with these proteins, we then measured whether inactivation of the helicase impairs RECQL4's ability to interact with CtIP and MRE11. RECQL4-KM-3xFLAG was pulled down with YFP-MRE11 to the same extent as RECQL4-3xFLAG (Figure 5C). Similarly, helicase-dead RECQL4 also interacted with CtIP as well as WT RECQL4 did (Figure 5D). These findings suggest that inactivation of the helicase domain of RECQL4 does not affect the interaction between RECQL4 and MRE11 or CtIP. Additionally, we found that both helicase-dead and WT RECQL4 proteins were able to stimulate the nuclease activity of MRN on closed circle single-strand PhiX174 DNA (Figure 5E). Taken together, the DNA unwinding activity of RECQL4 is required to promote DNA end resection and HR repair.

RPA-Mediated Displacement of RECQL4 from ssDNA

Unlike BLM and WRN, RECQL4 remains at DSB sites for only a short time (Singh et al., 2010), suggesting that it falls off or is dis-

placed. After DNA end resection, RPA coats the ssDNA tails for protection, which supported by the observation that RPA recruitment to DSBs increases continuously in 1 hr (Figure S5A), as previously reported (Costelloe et al., 2012). Thus, we used an in vitro RECQL4 displacement assay to determine whether RPA could remove RECQL4 from ssDNA. Biotin-labeled ssDNA, dsDNA, or 3' tailed dsDNA substrates were first incubated with RECQL4 and then with either RPA or BSA. RPA-mediated RECQL4 displacement was detected by visualization of RECQL4 in the supernatant (Figure 5F). Consistent with previous findings (Jensen et al., 2012; Keller et al., 2014), RECQL4 binds to ssDNA, dsDNA, and 3' tailed dsDNA substrates (Figure 5F). RPA prefers to bind DNA substrates with longer ssDNA (Figures S5C and S5D). When RPA was added to the RECQL4 coated 80-nt-long ssDNA G80, RECQL4 was displaced as RPA bound to this substrate (Figure 5F). A similar phenomenon was observed using GC40 and GC60 DNA substrates, which contain 40 or 20 nucleotide 3' tails, respectively (Figure 5F). However, very little RECQL4 was replaced by RPA from the blunt-ended dsDNA GC80 or from the 6-nt-tailed dsDNA G80/C74 (Figure 5F). BSA, our negative control, did not displace RECQL4 from the tested DNA substrates (Figure 5F). Together, these data imply that RPA can displace RECQL4 from ssDNA or 3' tailed dsDNA in vitro but not duplex DNA.

DISCUSSION

DNA end resection generates 3' tailed ssDNA, which is critical for launching HR repair. The MRN complex initiates 5' end resection with CtIP, and then extensive resection is carried out by the nucleases EXO1 or DNA2 in two alternative pathways (Cejka, 2015; Symington, 2014). In the present work, we establish that RECQL4 is required for robust DNA end resection by regulating the interaction between MRN and CtIP and further that the helicase activity of RECQL4 is required for the process. We show that depletion of RECQL4 results in loss of HR repair as a result of diminished 5' resection. IR enhances the physical interaction of RECQL4 with MRN and CtIP. The nuclease activity of MRE11 regulates the retention of RECQL4 at DSBs, and RECQL4 promotes recruitment of CtIP, as well as downstream players like BLM, DNA2, and EXO1, which participate in the extensive resection step of HR. Thus, this work ascribes a hitherto unrecognized role for RECQL4 as an important regulator of DNA end resection in HR repair.

The data presented here indicate that rapid recruitment of RECQL4 to laser-induced DSBs depends on prior recruitment of MRN while RECQL4 is not required for recruitment of MRN to IR-induced DSBs. Additionally, RECQL4 interacts physically with the MRN complex in living cells and under cell-free conditions in vitro (Figures 2A–2D). RECQL4 and MRN function in the same pathway during 5' end resection in HR repair (Figures 2H and 2I). These findings suggest that RECQL4 functions downstream of MRN in HR DSBR. Combining these findings with the previous reports that both MRE11 and RECQL4 immediately gather at laser-induced DSB (Haince et al., 2008; Singh et al., 2010), it is likely that MRN and RECQL4 act sequentially and cooperatively at DSBs to promote 5' end resection at DNA DSBs.

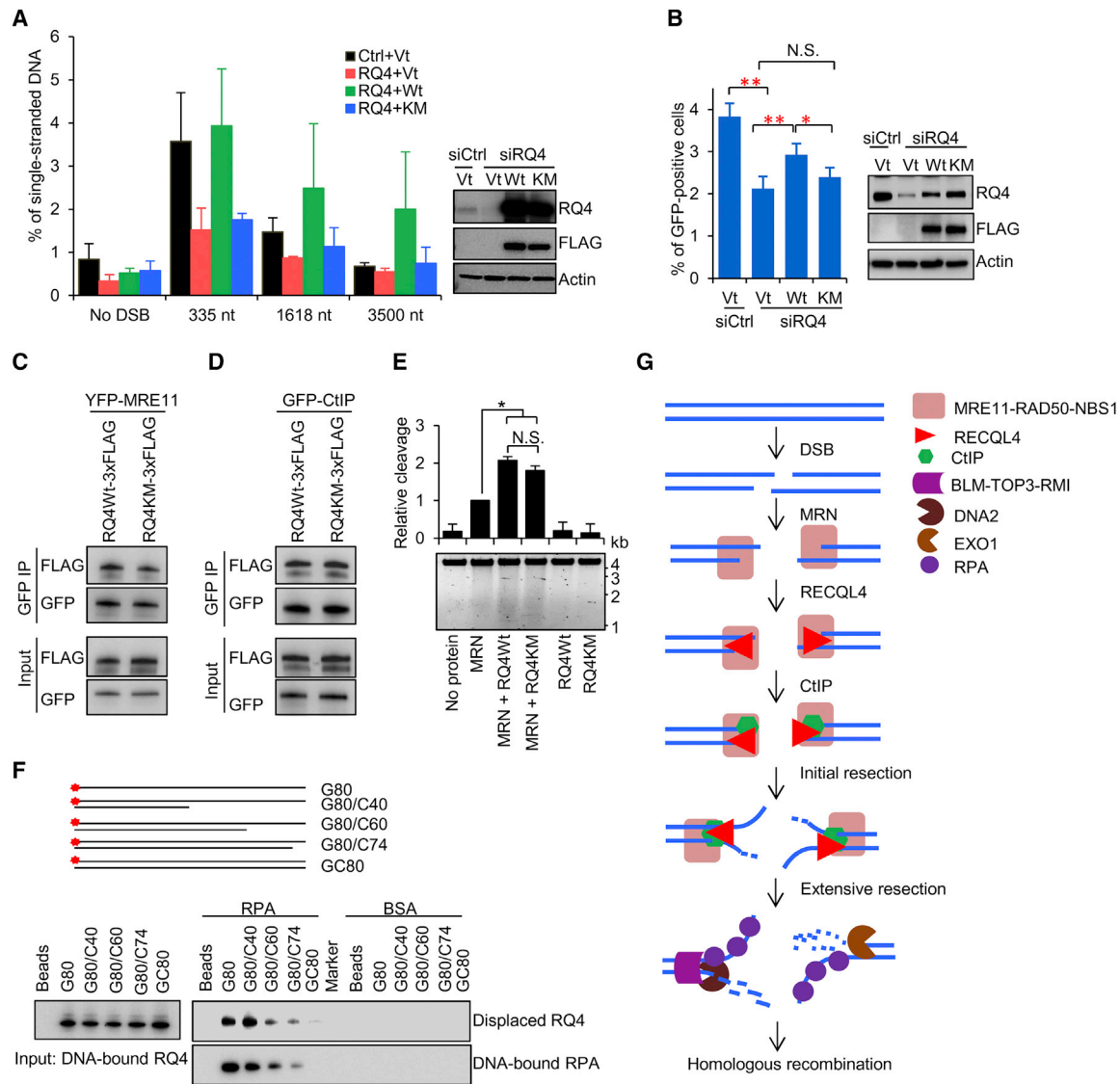


Figure 5. Helicase Activity of RECQL4 Is Required for RECQL4 to Function in DNA Resection and HR Repair

(A) Quantification of the ssDNA generated from resection at DSB1 after endogenous RECQL4 depletion of AID-DlvA U2OS cells but complementation with RECQL4-WT-3xFLAG or RECQL4-KM-3xFLAG. Data presented are mean \pm SEM from three biological repeats.

(B) HR repair assay after endogenous RECQL4 depletion in DR-GFP U2OS cells expressing wild-type or helicase dead mutant RECQL4. Error bars represent SEM from four independent experiments. N.S., no significance.

(C and D) Pull-down assay using YFP-MRE11 (C) or GFP-CtIP (D) with RQ4Wt-3xFLAG and RQ4KM-3xFLAG in vitro.

(E) Both WT and helicase-dead mutant RECQL4 significantly stimulate nuclease of MRN on closed circular single-strand PhiX174 DNA. The concentration of MRN, RECQL4 and RQ4KM were 20 nM. Error bars represent SEM from three repeats with p value by Student's t test.

(F) RPA displaces RECQL4 from ssDNA. Various substrates as shown were pre-incubated with RECQL4 then RPA or BSA were added to compete off RECQL4. Detection of displaced RECQL4 and DNA-bound RPA were visualized by western blotting.

(G) Model showing RECQL4's role in DNA end resection of HR-mediated repair. MRN complex recognizes and binds to DSBs and recruits RECQL4 to the sites of damage. In turn, RECQL4 promotes the stable recruitment of CtIP to DSBs and performs unwinding at the DNA ends thereby promoting resection.

See also Figure S5.

Retention of RECQL4 at DSBs is dramatically reduced in the presence of mirin (Figure 2G). Mirin inhibits MRE11 exonuclease and also represses MRN-dependent ATM activation (Dupré et al., 2008). After recruitment by MRN, RECQL4 is likely retained at DSBs by the resected DNA, which depends on the exonuclease activity of MRE11. Meanwhile, RECQL4 stimulates the

nuclease activity of MRN in vitro and promotes MRN-CtIP complex formation after IR, which is required for initiation of DNA resection (Sartori et al., 2007; You et al., 2009). Therefore, it is possible that RECQL4 promotes MRE11-mediated resection at a limited level, which further stimulates retention of RECQL4 at DSBs. Meanwhile, initially resected DNA also leads to limited

ATR activation and ATR-dependent phosphorylation of CtIP, and in turn promotes stable chromatin association of CtIP for robust resection (Peterson et al., 2013). With 5' resection ongoing, RPA binds to long ssDNA and disassociates RECQL4. Therefore, MRN, RECQL4, CtIP, and checkpoint kinases can function in a feedback loop during DNA end processing at DSBs.

Recruitment of CtIP to DSBs depends on MRN and ATM (You et al., 2009). MRN is recruited earlier than CtIP to DSBs; therefore, MRN may not directly recruit CtIP (You et al., 2009). This study reports that RECQL4 physically interacts with CtIP and promotes stable recruitment of CtIP to DSBs. However, the kinetics of the accumulation of CtIP differs from that of RECQL4. CtIP reaches its peak of abundance at laser-induced DSBs around 15 min after microirradiation (Figure 3G), as previously reported (Wang et al., 2013; You et al., 2009). However, RECQL4 only needs about 1 min to reach its recruitment peak (Figure 2G). Therefore, it is possible that RECQL4 recruits CtIP directly to initiate DNA resection, which further promotes more recruitment of CtIP due to checkpoint activation. RECQL4 also facilitates formation of an MRN-CtIP complex in vivo after IR (Figure 3H). In summary, the data are consistent with the model that RECQL4 promotes CtIP recruitment to DSBs and thereby directly promotes end processing and HR-dependent DSB repair.

BLM/DNA2 and EXO1 are required for extensive 5'-end-resection step during HR-dependent DSB repair (Symington, 2014). In contrast to RECQL4, BLM appears to play a somewhat more complex role during HR in human cells, possibly at two steps, once during end processing and a second time during dissolution of Holliday junctions together with TopoII α /RMI1/2 (Croteau et al., 2014). Here, we show that RECQL4 plays a role in recruiting and retaining BLM and EXO1 at DSBs and that RECQL4 acts in the same pathway as BLM and EXO1 (Figure 4). Since MRE11 and CtIP are required to recruit/retain BLM/EXO1 at DSBs (Eid et al., 2010; Truong et al., 2013; Wang et al., 2013), this lack of retention of BLM/EXO1 on DSBs in RECQL4-depleted cells is probably a consequence of the inhibition of CtIP recruitment caused by loss of RECQL4. Another possibility is that RECQL4 directly recruits BLM and EXO1 in order to switch from initial resection to extensive resection.

RECQL4 is unlikely to be directly involved in the extensive resection step with DNA2/BLM or EXO1, although it interacts with all of them. Short retention of RECQL4 at DSBs reduces the possibility that RECQL4 works together with DNA2/BLM or EXO1 in extensive resection. Second, BLM, but not RECQL4, specifically stimulates the nuclease activities of both DNA2 and EXO1 in vitro (Nimonkar et al., 2008, 2011). Moreover, RPA displaces RECQL4 from ssDNA (Figure 5F). However, it is possible that RECQL4 after interacting with MRN and CtIP remains bound to the ssDNA long enough to interact with DNA2/BLM and EXO1 and promotes their recruitment and chromatin association, which may activate the switch from initial resection to extensive resection.

Here, we found that depletion of either BLM or EXO1 reduces DNA resection and HR (Figure 4). In yeast, absence of Exo1 reduced resection 1–5 kb from the DSB (Llorente and Symington, 2004; Mimitou and Symington, 2008). Dysfunction of sgs1 also markedly reduced the DNA resection rate and efficiency (Zhu et al., 2008). However, co-depletion of sgs1 and exo1

caused more dramatic loss of DNA resection (Mimitou and Symington, 2008; Zhu et al., 2008). In human cells, depletion of EXO1, DNA2, or BLM reduced 5' DNA resection in U2OS cells (Grabarz et al., 2013; Gravel et al., 2008; Myler et al., 2016; Tomimatsu et al., 2012; Zhou et al., 2014). DNA2/BLM (Dna2/sgs1) and EXO1 can compensate for each other in extensive resection (Symington, 2014). However, dysfunction of either pathway could affect efficiency of DNA resection in vivo.

The helicase-dead mutant RECQL4-KM did not rescue the loss of either DNA end resection or HR repair (Figure 5), demonstrating that the helicase activity of RECQL4 is required for DNA end resection and HR repair. RECQL4-KM was generated by replacing lysine 508 in the Walker A motif of the SFII helicase domain with methionine, and the mutation eliminates 3'-5' DNA unwinding activity and ATPase of RECQL4 but not its annealing activity (Rossi et al., 2010). The expression of RECQL4-KM only partially restored the ability of RECQL4 to prevent cellular senescence in primary human fibroblasts with depletion of the endogenous RECQL4, indicating the importance of helicase activity in vivo (Lu et al., 2014a). However, the helicase activity is neither involved in the physical interaction between RECQL4 and MRN and CtIP, nor in the stimulation of the nuclease activity of MRN. In vivo DSBs are complex due to chromatin organization and DSB binding proteins competing at the site. RECQL4 has several activities. The failure of RECQL4-KM to rescue the resection and HR in endogenous RECQL4-depleted cells may reflect that it acts as a dominant-negative. RECQL4 can unwind dsDNA, dsDNA with 3' overhang (not 5' overhang), bubble-structured dsDNA, Y-structured duplex, and D-loop (Ghosh et al., 2012; Rossi et al., 2010; Xu and Liu, 2009). Thus, these activities might also help resolve secondary structures near the DNA ends to facilitate the initiation or extensive steps of DNA end resection.

A model for 5' DNA resection is presented in Figure 5G, which highlights the role of RECQL4 in initiating 5' end resection at a nascent DSB. DSB arise due to endo- or exogenous insults, which are first sensed by the MRN complex. RECQL4 is recruited by MRN, and RECQL4 possibly promotes a limited resection with MRN, which also promotes retention of RECQL4 at DSB. The MRN-RECQL4 complex then promotes recruitment of CtIP to DSBs. After CtIP enters the complex, the nuclease activity of MRN is greatly stimulated (Sartori et al., 2007). The resultant short ssDNA strands then may also promote greater retention of RECQL4 and CtIP. This feedback loop would then facilitate recruitment of proteins involved in the extensive end resection step like BLM/DNA2 and EXO1. We are proposing that an activity of RECQL4 may remove DNA secondary structure barriers near the ends of the DNA to promote MRN-mediated DNA resection. After some length of resection, RPA binds the ssDNA and promotes displacement of RECQL4 allowing it to be recycled for use at other dsDNA break sites.

EXPERIMENTAL PROCEDURES

Cell Culture, Knockdown, DNA Transfection, γ Radiation, and Survival Assay

U2OS, HEK293T, and HeLa cell lines were cultured in DMEM medium with 10% fetal bovine serum (Sigma-Aldrich), 1 \times penicillin/streptomycin (Gibco). All cells were cultured in an atmosphere of 5% CO₂ at 37°C. Lentivirus-mediated small hairpin RNA (shRNA) knockdown and siRNA knockdown were

performed as reported (Lu et al., 2014a). The sequences of siRNA and shRNA are listed in Table S2. Polyplus JetPrime was used for DNA transfection. γ rays were generated using a cesium-137 source (Gammacell Exactor 40, Best Theratronics). Radiation dose is 10 Gy, and post-irradiation recovery time is indicated in the figure legends. For the colony formation assay, cells were irradiated and then stained with 2% methylene blue in 5% ethanol 10 days after IR. The colonies with over 50 cells were counted. The results are presented as mean \pm SEM from three independent experiments with p value by Student's t test.

Laser-Induced DNA Damage and Real-Time Recruitment of Fluorescence Proteins

Laser-induced DSB and the recruitment of GFP-RECQL4, GFP-CtIP, GFP-BLM, GFP-EXO1, and GFP-RPA were performed as described (Singh et al., 2010). For mirin treatment, U2OS cells expressing GFP-RECQL4 were pre-incubated with 100 μ M mirin for 4 hr before laser microirradiation. The results are presented as mean \pm SEM with p value by Student's t test.

HR Assay

RECQL4 and other target proteins were knocked down by siRNA in the DR-GFP U2OS cells (a gift from Prof. Xiaofan Wang), and, 72 hr after siRNA transfection, the HR assay was performed as reported (Pierce et al., 1999; Wang et al., 2014). The siRQ4-resistant plasmids, pCMVTag4A-RQ4-siR and pCMVTag4A-RQ4KM-siR, expressing 3xFLAG-tagged wild-type RECQL4 and helicase-dead mutant RECQL4KM, respectively, were generated by PCR with primers RQ4-siR-PF and RQ4-siR-PR (Table S2). 0.5 μ g vector, pCMVTag4A-RQ4-siR, or pCMVTag4A-RQ4KM-siR were transfected into 5×10^5 siRQ4-treated DR-GFP U2OS cells. 24 hr later, cells were then processed for HR assay. The results are presented as mean \pm SEM from three independent experiments with p value by Student's t test.

5' Resection Assay

In vivo 5' end resection was measured in AID-DIVA U2OS cells (a gift from Dr. Gaëlle Legube), as previously described (Zhou et al., 2014). For the rescue assay, 5 μ g pCMVTag4A-RQ4-siR, pCMVTag4A-RQ4KM-siR, or control vector was transfected into 2×10^6 RECQL4 siRQ4-treated cells. 45 hr later, cells were treated with 4-OHT and then processed for the resection assay. At least three biological repeats were performed, and data are presented as the mean \pm SEM.

Subcellular Fractionation, Western Blotting, and Immunofluorescence Microscopy

Subcellular fractions were isolated using a subcellular protein fractionation kit (Thermo Fisher Scientific) according to the manufacturer's instructions, and the resultant fractions were analyzed with western blotting. Western blot and immunofluorescence microscopy were performed as previously described (Lu et al., 2014a). Antibodies used in this study are listed in the Supplemental Experimental Procedures.

Protein Purification

The MRE11-RAD50-NBS1 complex was purified from insect cells as previously described (Cheng et al., 2004). Purification of recombinant RECQL4 and helicase-dead mutant RECQL4-KM were performed as described (Rossi et al., 2010). RPA purification was performed as described (Henricksen et al., 1994). The details of CtIP purification and 3xFLAG-tagged RECQL4 and its truncation fragments are provided in the Supplemental Experimental Procedures.

IP, Pull-Down Assay, Silver Staining, and Protein Identification

Control and γ -irradiated cells were incubated for 10 min and then sonicated on ice in IP Lysis Buffer 2 containing 40 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM $MgCl_2$, 0.2% NP-40, 0.4% Triton 100, 1 \times protease inhibitor cocktail (Thermo Fisher Scientific), 1 \times phosphatase inhibitor cocktail 2 and 3 (Sigma-Aldrich), and 20 U/ml benzonase (Novagen). 50 μ g/ml ethidium bromide was added in lysate where it was indicated. For coIP with RECQL4 antibody, 2 mg protein was incubated with 2 μ g of RECQL4 antibody (Lu et al., 2014a) or normal rabbit immunoglobulin G (IgG) (Thermo Fisher Scientific). For FLAG IP, the cell lysate

was incubated with M2 FLAG-magnetic beads (Sigma-Aldrich). For GFP IP, GFP-TRAP beads (ChromoTek) were used to capture GFP-RECQL4 or YFP-MRE11. The beads were washed with cold washing buffer 4 (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.2% Triton X-100) five times and then subjected to western blotting, silver staining, or mass spectrometry analysis by Harvard Taplin Mass Spectrometry Facility. The details of mass spectrometry are listed in Table S1.

For in vitro IP, purified RECQL4 was incubated with recombinant MRN complex or CtIP in Binding Buffer 1 (20 mM Tris-HCl [pH 7.4], 100 mM NaCl, 0.2% Triton X-100) for 2 hr at 4°C and then incubated with anti-RECQL4 antibody or normal rabbit IgG. After washing with Binding Buffer, the proteins remained on the beads were analyzed by western blotting.

Nuclease Assay

Nuclease assays were carried out with 20 nM MRN or nuclease-dead MRN-ND in the presence of 20 nM wild-type RECQL4 or helicase-dead RECQL4 RQ4KM on 50 ng closed circular single-stranded PhiX174 DNA in the resection buffer containing 20 mM MOPS (pH 7.2), 1 mM DTT, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM ATP, as previously described (Sartori et al., 2007). After 3 hr incubation at 37°C, DNA was separated in 0.8% agarose gel, further stained with SYBR Gold, visualized with Chemidoc XRS+ system (Bio-Rad) and quantified with Bio-Rad Image Lab (v.3.0). Data were presented as mean \pm SEM from three repeats.

Displacement Assay

A biotin-labeled oligonucleotide (G80) was annealed with C80, C74, C60, and C40 and resulted in dsDNA GC80 and 3' tailed dsDNA GC74, GC60, and GC40, respectively (see the sequences in Table S2). RECQL4 (200 nM) was incubated with 20 nM DNA substrates bound to M280-streptavidin beads (Life Technologies) in the binding buffer (20 mM HEPES [pH 7.4], 100 mM NaCl, 1 mM $MgCl_2$, 0.1% Triton X-100, and 200 ng/ml BSA) at room temperature (RT) for 15 min. After washing with the binding buffer, the beads were incubated with 200 nM RPA or 200 ng/ml BSA at RT for 15 min. The supernatants and beads were then collected for detecting displaced RECQL4 and DNA-bound RPA by western blotting.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2016.05.079>.

AUTHOR CONTRIBUTIONS

Conceptualization: H.L., R.A.S., D.L.C., and V.A.B.; Methodology: H.L. and R.A.S.; Investigation: H.L. and R.A.S.; Writing—Original Draft, H.L. and R.A.S.; Review & Editing, H.L., R.A.S., L.J.R., P.C., D.L.C., and V.A.B.; Funding Acquisition, V.A.B.; Resources, G.K., R.A., L.J.R., and P.C.; Supervision, D.L.C. and V.A.B.

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REFERENCES

- Aparicio, T., Baer, R., and Gautier, J. (2014). DNA double-strand break repair pathway choice and cancer. *DNA Repair (Amst.)* 19, 169–175.
- Aymard, F., Bugler, B., Schmidt, C.K., Guillo, E., Caron, P., Briois, S., Iacovoni, J.S., Daburon, V., Miller, K.M., Jackson, S.P., and Legube, G. (2014). Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 21, 366–374.
- Bunting, S.F., Callén, E., Wong, N., Chen, H.T., Polato, F., Gunn, A., Bothmer, A., Feldhahn, N., Fernandez-Capetillo, O., Cao, L., et al. (2010). 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 141, 243–254.
- Cannavo, E., and Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. *Nature* 514, 122–125.
- Cejka, P. (2015). DNA end resection: nucleases team up with the right partners to initiate homologous recombination. *J. Biol. Chem.* 290, 22931–22938.
- Cejka, P., Cannavo, E., Polaczek, P., Masuda-Sasa, T., Pokharel, S., Campbell, J.L., and Kowalczykowski, S.C. (2010). DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. *Nature* 467, 112–116.
- Chen, L., Nievera, C.J., Lee, A.Y., and Wu, X. (2008). Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *J. Biol. Chem.* 283, 7713–7720.
- Chen, H., Lisby, M., and Symington, L.S. (2013). RPA coordinates DNA end resection and prevents formation of DNA hairpins. *Mol. Cell* 50, 589–600.
- Cheng, W.H., von Kobbe, C., Opresko, P.L., Arthur, L.M., Komatsu, K., Seidman, M.M., Carney, J.P., and Bohr, V.A. (2004). Linkage between Werner syndrome protein and the Mre11 complex via Nbs1. *J. Biol. Chem.* 279, 21169–21176.
- Costelloe, T., Louge, R., Tomimatsu, N., Mukherjee, B., Martini, E., Khadaroo, B., Dubois, K., Wiegant, W.W., Thierry, A., Burma, S., et al. (2012). The yeast Fun30 and human SMARCA1 chromatin remodellers promote DNA end resection. *Nature* 489, 581–584.
- Croteau, D.L., Popuri, V., Opresko, P.L., and Bohr, V.A. (2014). Human RecQ helicases in DNA repair, recombination, and replication. *Annu. Rev. Biochem.* 83, 519–552.
- Dupré, A., Boyer-Chatenet, L., Sattler, R.M., Modi, A.P., Lee, J.H., Nicolette, M.L., Kopelovich, L., Jasin, M., Baer, R., Paull, T.T., and Gautier, J. (2008). A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex. *Nat. Chem. Biol.* 4, 119–125.
- Eid, W., Steger, M., El-Shemerly, M., Ferretti, L.P., Peña-Díaz, J., König, C., Valtorta, E., Sartori, A.A., and Ferrari, S. (2010). DNA end resection by CtIP and exonuclease 1 prevents genomic instability. *EMBO Rep.* 11, 962–968.
- Fang, H., Nie, L., Chi, Z., Liu, J., Guo, D., Lu, X., Hei, T.K., Balajee, A.S., and Zhao, Y. (2013). RecQL4 helicase amplification is involved in human breast tumorigenesis. *PLoS ONE* 8, e69600.
- Ghosh, A.K., Rossi, M.L., Singh, D.K., Dunn, C., Ramamoorthy, M., Croteau, D.L., Liu, Y., and Bohr, V.A. (2012). RECQL4, the protein mutated in Rothmund-Thomson syndrome, functions in telomere maintenance. *J. Biol. Chem.* 287, 196–209.
- Grabarz, A., Guirouilh-Barbat, J., Barascu, A., Pennarun, G., Genet, D., Rass, E., Germann, S.M., Bertrand, P., Hickson, I.D., and Lopez, B.S. (2013). A role for BLM in double-strand break repair pathway choice: prevention of CtIP/Mre11-mediated alternative nonhomologous end-joining. *Cell Rep.* 5, 21–28.
- Gravel, S., Chapman, J.R., Magill, C., and Jackson, S.P. (2008). DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev.* 22, 2767–2772.
- Haince, J.F., McDonald, D., Rodrigue, A., Déry, U., Masson, J.Y., Hendzel, M.J., and Poirier, G.G. (2008). PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *J. Biol. Chem.* 283, 1197–1208.
- Henricksen, L.A., Umbricht, C.B., and Wold, M.S. (1994). Recombinant replication protein A: expression, complex formation, and functional characterization. *J. Biol. Chem.* 269, 11121–11132.
- Im, J.S., Ki, S.H., Farina, A., Jung, D.S., Hurwitz, J., and Lee, J.K. (2009). Assembly of the Cdc45-Mcm2-7-GINS complex in human cells requires the Ctf4/And-1, RecQL4, and Mcm10 proteins. *Proc. Natl. Acad. Sci. USA* 106, 15628–15632.
- Jensen, M.B., Dunn, C.A., Keijzers, G., Kulikowicz, T., Rasmussen, L.J., Croteau, D.L., and Bohr, V.A. (2012). The helicase and ATPase activities of RECQL4 are compromised by mutations reported in three human patients. *Aging (Albany, N.Y.)* 4, 790–802.
- Keller, H., Kiosze, K., Sachsenweger, J., Haumann, S., Ohlenschläger, O., Nuutinen, T., Syväoja, J.E., Görlach, M., Grosse, F., and Pospiech, H. (2014). The intrinsically disordered amino-terminal region of human RecQL4: multiple DNA-binding domains confer annealing, strand exchange and G4 DNA binding. *Nucleic Acids Res.* 42, 12614–12627.
- Llorente, B., and Symington, L.S. (2004). The Mre11 nuclease is not required for 5′ to 3′ resection at multiple HO-induced double-strand breaks. *Mol. Cell. Biol.* 24, 9682–9694.
- Lu, H., Fang, E.F., Sykora, P., Kulikowicz, T., Zhang, Y., Becker, K.G., Croteau, D.L., and Bohr, V.A. (2014a). Senescence induced by RECQL4 dysfunction contributes to Rothmund-Thomson syndrome features in mice. *Cell Death Dis.* 5, e1226.
- Lu, L., Jin, W., Liu, H., and Wang, L.L. (2014b). RECQ DNA helicases and osteosarcoma. *Adv. Exp. Med. Biol.* 804, 129–145.
- Mimitou, E.P., and Symington, L.S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455, 770–774.
- Myler, L.R., Gallardo, I.F., Zhou, Y., Gong, F., Yang, S.H., Wold, M.S., Miller, K.M., Paull, T.T., and Finkelstein, I.J. (2016). Single-molecule imaging reveals the mechanism of Exo1 regulation by single-stranded DNA binding proteins. *Proc. Natl. Acad. Sci. USA* 113, E1170–E1179.
- Nimonkar, A.V., Ozsoy, A.Z., Genschel, J., Modrich, P., and Kowalczykowski, S.C. (2008). Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc. Natl. Acad. Sci. USA* 105, 16906–16911.
- Nimonkar, A.V., Genschel, J., Kinoshita, E., Polaczek, P., Campbell, J.L., Wyman, C., Modrich, P., and Kowalczykowski, S.C. (2011). BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev.* 25, 350–362.
- Niu, H., Chung, W.H., Zhu, Z., Kwon, Y., Zhao, W., Chi, P., Prakash, R., Seong, C., Liu, D., Lu, L., et al. (2010). Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature* 467, 108–111.
- Park, S.J., Lee, Y.J., Beck, B.D., and Lee, S.H. (2006). A positive involvement of RecQL4 in UV-induced S-phase arrest. *DNA Cell Biol.* 25, 696–703.
- Peterson, S.E., Li, Y., Wu-Baer, F., Chait, B.T., Baer, R., Yan, H., Gottesman, M.E., and Gautier, J. (2013). Activation of DSB processing requires phosphorylation of CtIP by ATR. *Mol. Cell* 49, 657–667.
- Pierce, A.J., Johnson, R.D., Thompson, L.H., and Jasin, M. (1999). XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev.* 13, 2633–2638.
- Prakash, R., Zhang, Y., Feng, W., and Jasin, M. (2015). Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. *Cold Spring Harb. Perspect. Biol.* 7, a016600.
- Rossi, M.L., Ghosh, A.K., Kulikowicz, T., Croteau, D.L., and Bohr, V.A. (2010). Conserved helicase domain of human RecQ4 is required for strand annealing-independent DNA unwinding. *DNA Repair (Amst.)* 9, 796–804.
- Sangrithi, M.N., Bernal, J.A., Madine, M., Philpott, A., Lee, J., Dunphy, W.G., and Venkataraman, A.R. (2005). Initiation of DNA replication requires the RECQL4 protein mutated in Rothmund-Thomson syndrome. *Cell* 121, 887–898.
- Sartori, A.A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., and Jackson, S.P. (2007). Human CtIP promotes DNA end resection. *Nature* 450, 509–514.

- Shamanna, R.A., Singh, D.K., Lu, H., Mirey, G., Keijzers, G., Salles, B., Croteau, D.L., and Bohr, V.A. (2014). RECQ helicase RECQL4 participates in non-homologous end joining and interacts with the Ku complex. *Carcinogenesis* 35, 2415–2424.
- Shao, R.G., Cao, C.X., Zhang, H., Kohn, K.W., Wold, M.S., and Pommier, Y. (1999). Replication-mediated DNA damage by camptothecin induces phosphorylation of RPA by DNA-dependent protein kinase and dissociates RPA:DNA-PK complexes. *EMBO J.* 18, 1397–1406.
- Shibata, A., Moiani, D., Arvai, A.S., Perry, J., Harding, S.M., Genois, M.M., Maity, R., van Rossum-Fikkert, S., Kertokallio, A., Romoli, F., et al. (2014). DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. *Mol. Cell* 53, 7–18.
- Siitonen, H.A., Sotkasiira, J., Biervliet, M., Benmansour, A., Capri, Y., Cormier-Daire, V., Crandall, B., Hannula-Jouppi, K., Hennekam, R., Herzog, D., et al. (2009). The mutation spectrum in RECQL4 diseases. *Eur. J. Hum. Genet.* 17, 151–158.
- Singh, D.K., Karmakar, P., Aamann, M., Schurman, S.H., May, A., Croteau, D.L., Burks, L., Plon, S.E., and Bohr, V.A. (2010). The involvement of human RECQL4 in DNA double-strand break repair. *Aging Cell* 9, 358–371.
- Singh, D.K., Popuri, V., Kulikowicz, T., Shevelev, I., Ghosh, A.K., Ramamoorthy, M., Rossi, M.L., Janscak, P., Croteau, D.L., and Bohr, V.A. (2012). The human RecQ helicases BLM and RECQL4 cooperate to preserve genome stability. *Nucleic Acids Res.* 40, 6632–6648.
- Stracker, T.H., Carson, C.T., and Weitzman, M.D. (2002). Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* 418, 348–352.
- Su, Y., Meador, J.A., Calaf, G.M., Proietti De-Santis, L., Zhao, Y., Bohr, V.A., and Balajee, A.S. (2010). Human RecQL4 helicase plays critical roles in prostate carcinogenesis. *Cancer Res.* 70, 9207–9217.
- Symington, L.S. (2014). End resection at double-strand breaks: mechanism and regulation. *Cold Spring Harb. Perspect. Biol.* 6, a016436.
- Tomimatsu, N., Mukherjee, B., Deland, K., Kurimasa, A., Bolderson, E., Khanna, K.K., and Burma, S. (2012). Exo1 plays a major role in DNA end resection in humans and influences double-strand break repair and damage signaling decisions. *DNA Repair (Amst.)* 11, 441–448.
- Truong, L.N., Li, Y., Shi, L.Z., Hwang, P.Y., He, J., Wang, H., Razavian, N., Berns, M.W., and Wu, X. (2013). Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proc. Natl. Acad. Sci. USA* 110, 7720–7725.
- Wang, H., Shi, L.Z., Wong, C.C., Han, X., Hwang, P.Y., Truong, L.N., Zhu, Q., Shao, Z., Chen, D.J., Berns, M.W., et al. (2013). The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. *PLoS Genet.* 9, e1003277.
- Wang, Q., Goldstein, M., Alexander, P., Wakeman, T.P., Sun, T., Feng, J., Lou, Z., Kastan, M.B., and Wang, X.F. (2014). Rad17 recruits the MRE11-RAD50-NBS1 complex to regulate the cellular response to DNA double-strand breaks. *EMBO J.* 33, 862–877.
- Xu, X., and Liu, Y. (2009). Dual DNA unwinding activities of the Rothmund-Thomson syndrome protein, RECQ4. *EMBO J.* 28, 568–577.
- Xu, X., Rochette, P.J., Feyissa, E.A., Su, T.V., and Liu, Y. (2009). MCM10 mediates RECQ4 association with MCM2-7 helicase complex during DNA replication. *EMBO J.* 28, 3005–3014.
- You, Z., Shi, L.Z., Zhu, Q., Wu, P., Zhang, Y.W., Basilio, A., Tonnu, N., Verma, I.M., Berns, M.W., and Hunter, T. (2009). CtIP links DNA double-strand break sensing to resection. *Mol. Cell* 36, 954–969.
- Yuan, J., and Chen, J. (2009). N terminus of CtIP is critical for homologous recombination-mediated double-strand break repair. *J. Biol. Chem.* 284, 31746–31752.
- Zhou, Y., Caron, P., Legube, G., and Paull, T.T. (2014). Quantitation of DNA double-strand break resection intermediates in human cells. *Nucleic Acids Res.* 42, e19.
- Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. (2008). Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134, 981–994.